

ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: Biochemical Markers

Kjell Oberg^a Anne Couvelard^b Gianfranco Delle Fave^c David Gross^d
Ashley Grossman^e Robert T. Jensen^f Ulrich-Frank Pape^g Aurel Perren^h
Guido Rindiⁱ Philippe Ruszniewski^j Jean-Yves Scoazec^k Staffan Welin^a
Bertram Wiedenmann^l Diego Ferone^m all other Antibes Consensus
Conference participants

^aDepartment of Endocrine Oncology, Uppsala University Hospital, Uppsala, Sweden; ^bDepartment of Pathology, Hospital Beaujon, Clichy, France; ^cDepartment of Digestive and Liver Disease, Ospedale Sant'Andrea, Rome, Italy; ^dDepartment of Endocrinology and Metabolism, Hadassah University Hospital, Jerusalem, Israel; ^eOxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, University of Oxford, Oxford, UK; ^fDigestive Diseases Branch, National Institutes of Health, Bethesda, MD, USA; ^gDepartment of Internal Medicine, Charité University of Berlin, Berlin, Germany; ^hDepartment of Pathology, University Hospital Zurich, Zurich, Switzerland; ⁱInstitute of Anatomic Pathology, Policlinico A. Gemelli, Università Cattolica del Sacro Cuore, Rome, Italy; ^jDepartment of Gastroenterology, Beaujon Hospital, Clichy, and ^kDepartment of Biopathology, Gustave Roussy Institute, Faculty of Medicine Paris Sud, Paris, France; ^lDepartment of Hepatology and Gastroenterology, Charité University Medicine, Berlin, Germany; ^mEndocrinology Unit, Department of Internal Medicine and Medical Specialties (DiMI), Center of Excellence for Biomedical Research (CEBR), IRCCS AOU San Martino-IST, University of Genova, Genova, Italy

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Abstract

Biomarkers have been the mainstay in the diagnosis and follow-up of patients with neuroendocrine tumors (NETs) over the last few decades. In the beginning, secretory products from a variety of subtypes of NETs were regarded as biomarkers to follow during diagnosis and treatment: serotonin for small intestinal (SI) NETs, and gastrin and insulin for pancreatic NETs. However, it became evident that a large number of NETs were so-called nonfunctioning tumors without

secreting substances that caused hormone-related symptoms. Therefore, it was necessary to develop so-called “general tumor markers.” The most important ones so far have been chromogranin A and neuron-specific enolase (NSE). Chromogranin A is the most important general biomarker for most NETs with a sensitivity and specificity somewhere between 60 and 90%. NSE has been a relevant biomarker for patients with high-grade tumors, particularly lung and gastrointestinal tract tumors. Serotonin and the breakdown product urinary 5-hydroxyindoleacetic acid (U-5-HIAA) is still an important marker for diagnosing and follow-up of SI NETs. Recently, 5-HIAA in plasma has been analyzed by high-performance liquid chromatography and fluorometric detection and has shown good agreement with U-5-HIAA anal-

ysis. In the future, we will see new tests including circulating tumor cells, circulating DNA and mRNA. Recently, a NET test has been developed analyzing gene transcripts in circulating blood. Preliminary data indicate high sensitivity and specificity for NETs. However, its precise role has to be validated in prospective randomized controlled trials which are ongoing right now.

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Introduction

Over the years, a number of general and specific circulatory biomarkers (Table 1) have been developed for the diagnosis and follow-up of patients with neuroendocrine tumors (NETs). The most important ones will be discussed in detail in the present paper. New biomarkers, such as circulating DNA, mRNA and circulating tumor cells, are currently under development; however, they are not ready for clinical routine use and have to be evaluated in prospective randomized trials.

Small Intestinal NETs

NETs originating from the small intestine (midgut) may result in functional symptoms due to the secretion of various peptides and hormones and most notably 5-hydroxytryptamine or serotonin. This is a tryptophan-derived biogenic amine involved in smooth muscle contraction, blood pressure regulation, and both peripheral and central nervous system neurotransmission. Approximately 2% of dietary tryptophan is converted into serotonin. Serotonin is synthesized and stored in enterochromaffin cells of the gastrointestinal tract (80% of total body serotonin content), in dense granules of platelets (storage only), and in the serotonergic neurons of the central nervous system. The urinary breakdown metabolite of serotonin is urinary 5-hydroxyindoleacetic acid (U-5-HIAA), which is particularly useful in the diagnosis and follow-up of NETs with carcinoid syndrome [1–4]. Serum measurements of serotonin are possible in these patients; however, large individual variations make them unreliable for diagnosis and in follow-up [3]. Universally, U-5-HIAA is the most frequently performed assay in the clinical setting of the carcinoid syndrome. Serum 5-HIAA may represent a future tool for diagnosing and follow-up of small intestinal (SI) NETs with carcinoid syndrome. A couple of recent trials have demonstrated a good agreement between measurements of U-5-HIAA and serum

5-HIAA [5–7]. Indeed, plasma and urine 5-HIAA displayed similar diagnostic sensitivities and specificities, warranting, however, further validation on larger patient populations. The carcinoid syndrome also has other mediators than serotonin, such as substance P and neurokinin A (tachykinins).

Performance of 5-HIAA in Diagnosis

The overall sensitivity and specificity of U-5-HIAA in the presence of the carcinoid syndrome is of the order of 70 and 90%, respectively [1, 2]. Midgut NETs are most liable to produce the carcinoid syndrome with U-5-HIAA elevation, thus attesting to a high specificity (>90%) in this setting. Foregut and hindgut NETs produce less serotonin than midgut tumors [1, 3]. U-5-HIAA levels may also depend on tumor volume and may be normal in patients with nonmetastatic tumors. Levels may be normal even in the presence of the carcinoid syndrome, particularly in subjects without diarrhea; however, this is a rare event [3]. In functional SI NETs, discriminating performances may vary depending on whether the cutoffs are high or low. Meijer et al. [1] demonstrated that a low-level U-5-HIAA cutoff value (2.8 mmol/mol creatinine) yielded 68% sensitivity and 89% specificity, whereas a higher cutoff (6.7 mmol/mol creatinine) improved specificity to 98% at the expense of a lower sensitivity (52%). Thus, in order to confidently exclude an SI NET, a low-level cutoff value may be preferred; to confirm the presence of an SI NET, a high-level cutoff value is better. Some patients with the carcinoid syndrome excrete nonhydroxylated indole acids, not measured as U-5-HIAA. There appears to be an inconstant correlation between the U-5-HIAA level and the clinical severity of the carcinoid syndrome; this may be related to a fluctuating release of serotonin from tumors, such that the correlation may not be reliable. The possibility of carcinoid syndrome associated with normal 5-HIAA levels could be explained by the presence of other circulating biologically active molecules, which may often be secreted or co-secreted in patients with lung and midgut NETs.

Recent data have examined U-5-HIAA as a prognostic factor in these patients: while interesting data have emerged, the expert group felt that data have not confirmed U-5-HIAA levels to be a *consistently reliable* prognostic factor in this disease. To illustrate this, two studies including 256 and 139 patients with SI NETs showed that while elevated U-5-HIAA levels were predictive of poor outcome at univariate analysis, this did not remain sig-

Table 1. General and specific biomarkers currently used for the management of patients with neuroendocrine tumors

General tumor markers	Related indications
Chromogranin A	Almost all NETs (follow-up, limited in diagnosis)
Neuron-specific enolase	Atypical carcinoids, lung NEC, microcytoma
Pancreatic polypeptide	Pancreatic NET
α -Subunit, β -hCG	Pancreatic, lung NET
Specific tumor markers	Related indications
Serotonin, 5-HIAA	Well differentiated NET
Gastrin	Zollinger-Ellison syndrome
Insulinoma	Insulin-secreting pancreatic NET
Glucagon, VIP, somatostatin	Well differentiated pancreatic NET
Catecholamines	Pheocromocytoma/paraganglioma
Calcitonin	Medullary thyroid cancer and pancreatic NET
PTHrp, ACTH, CRH, GHRH	Syndromes from (ectopic) mainly lung or pancreatic NET
NTpro-BPN (marker of ventricular dysfunction)	Carcinoid syndrome (carcinoid heart disease)

NET, neuroendocrine tumor; NEC, neuroendocrine carcinoma; 5-HIAA, 5-hydroxyindolacetic acid; VIP, vasoactive intestinal peptide; PTHrp, parathormone-related peptide; ACTH, adrenocorticotropin hormone; CRH, corticotropin-releasing hormone; GHRH, growth hormone-releasing hormone; NTpro-BPN, N-terminal pro-brain natriuretic peptide.

nificant at multivariate analysis [4, 8]. In another study examining 76 patients, those with persistent moderately increased U-5-HIAA levels (≤ 20 mmol/mol creatinine) had a more favorable outcome compared to those with greatly elevated levels [9]. A further study in a mixed tumor group including 119 patients (53 with SI NETs) interestingly found high U-5-HIAA to be an independent survival factor [10].

Assays for 5-HIAA

While several assays are available to measure U-5-HIAA (thin-layer chromatography, enzyme immunoassay, gas chromatography, and gas chromatography-mass spectrometry) [11–14], the use of high-performance liquid chromatography (HPLC) is most frequently employed. HPLC with electrochemical detection is currently recommended; however, automated assays [15] or those using mass spectrometry [14] may be available in some laboratories. Liquid chromatography tandem mass spectrometry assay appears to be a rapid assay with little necessity for repeat analyses because of chromatographic interference or dilutions [14]. A further automated method with on-line solid-phase extraction and HPLC and fluorometric detection has recently been shown to have in-

creased precision and faster throughput compared to the manual solvent extraction method [16]. Whatever technique is used, it should be performed in accredited laboratories. Serum 5-HIAA is analyzed by liquid chromatography tandem mass spectrometry assay [5–7].

Conditions for an Optimal Assay

Urine should be collected and measured in plastic containers. Acid should be added to ensure sterility and hence stability. The sample should be stored in a refrigerator until analysis. All the urine passed over 24 h should be collected into the container, preferably by using a measuring jug. Collecting should be started at a defined time point following urination, and after that urine should be collected until the same time point the next day (a precise 24-h collection). Written instructions should be handed out including food and medication precautions (Table 2).

Care in Interpreting U-5-HIAA Levels

Intraindividual variation of U-5-HIAA is also possible and this variation may be high; therefore, 2 consecutive 24-h collections should be performed and the mean

Table 2. Factors interfering with measurements of urinary 5-hydroxyindole acetic acid

Foods	Drugs
<i>Factors producing false-positive results</i>	
Avocado	Acetaminophen
Banana	Acetanilide
Chocolate	Caffeine
Coffee	Fluorouracil
Eggplant (aubergine)	Guaifenesin
Pecan	L-DOPA
Pineapple	Melphalan
Plum	Mephenesin
Tea	Metamphetamine
Walnuts	Methocarbamol
	Methysergide maleate
	Phenmetrazine
	Reserpine
	Salicylates
<i>Factors producing false-negative results</i>	
None	Corticotropin
	p-chlorophenylalanine
	Chlorpromazine
	Heparin
	Imipramine
	Isoniazid
	Methenamine maleate
	Methyldopa

value of these 2 can be taken, especially when the collection is required for diagnosis. A single specimen may be sufficient for follow-up purposes. Certain comorbidities or associated disorders may have effects on the concentration of U-5-HIAA. Falsely low U-5-HIAA levels may be encountered in patients with renal impairment and those on hemodialysis. In addition, U-5-HIAA may be increased in untreated patients with malabsorption, who have increased urinary tryptophan metabolites. Such patients include those with gluten-sensitive enteropathy (celiac disease), tropical sprue, Whipple disease, intestinal stasis, and cystic fibrosis (chronic intestinal obstruction) [1, 17]; plasma 5-hydroxytryptamine, but not U-5-HIAA, has been elevated in diarrhea-predominant irritable bowel syndrome [18]. A small number of normal individuals may have elevated U-5-HIAA and, therefore, other objective findings should be used in conjunction with tumor marker analysis to support the diagnosis of an SI NET [19]. The following food substances are rich in dietary tryptophan and, therefore, patients should abstain from these for 3 days prior to urinary collection: plums, pineapples, bananas, eggplants (aubergines), to-

matoes, avocados, and walnuts [2, 20, 21]. Even certain medications may increase or decrease U-5-HIAA levels (Table 2).

Patients are frequently treated with somatostatin analogues and these are known to decrease levels of U-5-HIAA; where possible, assays for diagnostic purposes should be made in patients not on somatostatin analogues, while in the follow-up setting, comparisons should be performed in patients on stable or comparable doses. Recently, significantly elevated levels of U-5-HIAA have been confirmed as a negative predictor for overall survival, except when considered with other biomarkers and grading, suggesting its use to assess carcinoid syndrome without having a prognostic value [22].

Pancreatic NETs

Insulinoma: 72-h Fast

NETs secreting insulin are termed insulinomas and are almost exclusively intrapancreatic in nature. Excessive insulin secretion leading to hypoglycemia usually results in a combination of neurologic (diplopia, blurred vision, confusion, abnormal behavior and amnesia, seizures, coma, etc.) and autonomic (sweating, weakness, hunger, tremor, nausea, feelings of warmth, anxiety, palpitations) symptoms. Such symptoms are usually related to the degree of insulin-induced hypoglycemia, but may be nonspecific. Hypoglycemia-induced clinical signs are classically present in the early morning preprandial phase or may be exercise-induced. The diagnosis is suggested in the presence of: (1) symptoms of hypoglycemia; (2) glucose <2.2 mmol/L (40 mg/dL; others use a threshold of <3 mmol/L, 55 mg/dL); and (3) relief of symptoms with administration of glucose [23]. This is known as the Whipple triad. The 72-h fast is the gold standard for diagnosing insulinoma and relates to the integrity of the patient's endogenous suppression of insulin in the face of hypoglycemia. The fast attests to autonomous insulin secretion and the failure of appropriate insulin suppression in the presence of hypoglycemia. Factitious hypoglycemia secondary to the exogenous use of insulin is suspected on the finding of high (often very high) serum insulin in combination with suppression of C-peptide. Sulfonylureas and related insulin secretagogues result in a clinical picture similar to patients with insulinoma and may be diagnosed by a positive drug screen [24]. An overall approach to diagnosing and managing insulinoma has been provided elsewhere in another consensus statement [25].

Supervised 72-h Fast

This test has been verified as the gold standard in establishing a biochemical diagnosis of insulinoma [26]. Patients should be hospitalized in a specialist unit experienced in performing the test. A 72-h period is universally recognized as the most appropriate duration [25], although some groups have proposed a shorter fast of 48 h [27, 28]. Symptoms appear within 12 h for one-third of patients, within 24 h for 80%, within 48 h for 90%, and within 72 h for nearly 100% [29]. Absolute values of glucose and insulin are the most important variables and any measurable insulin is abnormal when blood glucose drops to 2.5 mmol/L (45 mg/dL). Assays used for the determination of insulin, proinsulin, C-peptide and β -hydroxybutyrate may vary but should be performed in accredited laboratories. Very occasionally, an insulinoma is only revealed by hypoglycemia induced by a mixed meal rather than fasting.

Patient Information Scheme

A detailed description of the fast should be provided to all patients with an information card to help in symptom identification. Patients should stay off all foods, except for plain water, black tea or coffee, and essential medications (particularly hypoglycemic agents; e.g., sulfonylureas).

Procedure

The timing of the 72-h fast is not critical – some teams prefer to perform the test early in the week when staffing levels may be higher, thus avoiding prolonging the test into the weekend. An oral glucose tolerance or mixed meal test can be performed before the fast. The patient should be monitored in a supervised environment and fasting should be accompanied by an intravenous line.

- Absolute blood (venous) determinations should be performed at least 2–4 times per day and when the patient describes symptoms. The test interpretation should be made using laboratory blood glucose assays; bedside measurements can be used in the presence of clinical symptoms to determine if more definitive measurements should be made.
- Blood should also be drawn for insulin measurement concurrently with glucose estimations, and assay for insulin and C-peptide when hypoglycemia is confirmed.
- β -Hydroxybutyrate (or urinary ketones) should be measured at the end of the test in order to confirm the validity of the fast. A low level of hydroxybutyrate in the presence of hypoglycemia confirms inappropriate insulin or insulin-like hormone secretion.

- A urinary assay for sulfonylureas should be performed as a specific request:
 - Not all drugs are detected, e.g., repaglinide [24]; false-positive results may also occur; e.g., on paracetamol.
 - The results need to be confirmed with the local laboratory.

Definition of Hypoglycemia

The endpoint of the test is documented hypoglycemia:

- Documented blood glucose levels 2.2 mmol/L (\approx 40 mg/dL; according to some <3 mmol/L, 55 mg/dL; levels may depend on age and sex).
- Concomitant insulin levels >6 μ U/L (≥ 36 pmol/L; ≥ 3 μ U/L by ICMA).
- A β -hydroxybutyrate level ≥ 2.7 mmol/L can be used as a surrogate marker to confirm the validity of the fast and inappropriate insulin suppression.
- A glucagon test immediately after 72-h fasting in patients without definite results has also been recommended.
- Exercise test immediately after 72-h fasting in patients without definitive results may be performed in a supervised setting.
- Use of a ratio of insulin to glucose to aid in the diagnosis is not recommended.

Gastrinoma (Zollinger-Ellison Syndrome)

Standards for the Diagnosis of a Gastrinoma: Secretin Test

The diagnosis of the Zollinger-Ellison syndrome (ZES) can be established by the demonstration of elevated fasting serum gastrin (FSG) in the presence of low gastric pH. FSG alone is not adequate to make the diagnosis of ZES because hypergastrinemia can be seen in patients with hypo- or achlorhydria associated with chronic atrophic fundus gastritis (e.g., pernicious anemia) and in other conditions with hyperchlorhydria (e.g., *Helicobacter pylori* infection, gastric outlet obstruction, renal failure, antral G-cell syndromes, short bowel syndrome, and retained antrum). In addition, the use of chronic proton pump inhibitors (PPIs) leads to high FSG levels and, therefore, gastrin provocative tests are needed to establish the diagnosis of ZES. Indeed, in a recent prospective analysis, up to two-thirds of gastrinoma patients were found to have FSG values being <10 -fold normal [30]. The gold standard is the secretin test [30–34]. This hormone, when given intravenously, provokes an increase in serum gas-

trin and, secondarily, in gastric acid secretion. The most reliable data concerning the secretin test have emanated from the National Institutes of Health (NIH) studies in patients with sporadic and multiple endocrine neoplasia type 1-associated gastrinomas [30–34]. Consensus guidelines have described the criteria used for establishing the diagnosis of gastrinoma [33]; however, according to the expert committee, acid output studies are available to only a limited number of groups (including those expert groups). For the NIH group, the secretin test was useful in diagnosing ZES regardless of the extent or locations of the tumor, the presence or absence of multiple endocrine neoplasia type 1 or the level of FSG (less than or greater than 1,000 pg/mL) [31]. In patients with fasting gastrin <1,000 pg/mL, the sensitivity of the secretin test using the criterion delta (increase from a prestimulation level) gastrin of ≥ 110 pg/mL was 93% (95% CI, 76–99) and for a delta gastrin of 200 pg/mL, sensitivity was 85% (95% CI, 66–96) ($p > 0.05$) [31]. The same group recently reported their prospective experience with gastrin provocative tests in 293 ZES patients from the NIH and compared them with 537 ZES patients from the literature and 462 non-ZES patients (again from the literature) [33]. This group established a delta gastrin of ≥ 120 pg/mL in patients with a <10-fold increase as having the highest sensitivity and specificity (94 and 100%, respectively) [33]. They also demonstrated the clear superiority of the secretin provocation test compared to the calcium test (94 vs. 62%). However, in ZES patients with a negative secretin test, the calcium provocation test may be helpful [33]. The expert group noted that certain groups had difficulty in obtaining secretin, hindering accurate diagnosis.

Indications for Gastrin Provocative Tests: Secretin Test

- The secretin test is performed to confirm a biochemical diagnosis of gastrinoma. The test may be repeated during the follow-up after curative surgery. FSG should be performed prior to the secretin test; if FSG >1,000 pg/mL, a secretin test is not necessary. When FSG lies between 200 and 1,000 pg/mL, a secretin test should be performed.
- The following conditions should also be documented:
 - Absence of fundic atrophic gastritis:
 - Antral and fundic biopsies (\pm serology for anti-parietal and intrinsic factor antibodies)
 - 24-h pH-metry (loss of diurnal pH course); basal acid output is recommended before and after secretin where possible; BAO >15 mmol/h is highly suggestive of diagnosis of ZES; a random pH anal-

ysis during upper gastrointestinal endoscopy was also suggested (this requires further evaluation)

- *Helicobacter pylori* testing
- Other conditions leading to high FSG should be considered including gastric outlet obstruction, renal failure, antral G-cell syndromes, short bowel syndrome, and retained gastric antrum.
- Treatment with PPIs interferes with basal FSG, as well as the secretin test [35].

Preparation for Secretin Test

- If possible, PPIs should be interrupted 10 days to 2 weeks prior to the test (PPIs for 2 weeks can be replaced by H₂ blockers); interruption of H₂ blockers for approximately 48 h prior to the test; however, interruption of all antisecretory medications should be individually adapted and patients should be warned of reappearance of symptoms and should have sufficient antisecretory medications to start should they become symptomatic; certain patients may have to be hospitalized during antisecretory therapy withdrawal.
- Heparinized vacutainers are used and should be labeled and placed in ice.

Secretin Test

- Patient fasting overnight, for 12–14 h
- Site indwelling intravenous cannula
- Kabi-secretin (2 U/kg body weight) is given by intravenous bolus
- Serum gastrin:
 - Baseline measured at –15 and –1 min before the test
 - 2, 5, 10, 15, 20, and 30 min after secretin
- Samples stored on ice (*immediate* transfer to laboratory)

Possible side effects of the secretin test include flush and an allergic reaction.

Interpretation of Results

- Delta gastrin of at least 200 pg/mL any time during the test is considered as positive.
- The NIH has recently published a delta gastrin of ≥ 120 pg/mL as having a high sensitivity and specificity (94 and 100%, respectively) [33].

General Biomarkers for NETs

Serum Chromogranin A

Chromogranin A (CgA) is an acid glycoprotein with 439 amino acids that is present in the secretory dense core

granules of most neuroendocrine cells [36]. The chromogranin family consists of at least 3 different water-soluble acidic glycoproteins (CgA, CgB, and secretogranin II, sometimes called chromogranin C). Upon stimulation, CgA and other peptide hormones and neuropeptides are released. CgA is also secreted from neuroendocrine-derived tumors including foregut, midgut and hindgut gastrointestinal NETs, pheochromocytomas, neuroblastomas, medullary thyroid carcinomas, some pituitary tumors, functioning and nonfunctioning pancreatic NETs, and other amine precursor uptake and decarboxylation tumors. CgA has also been widely used as an immunohistochemical marker in NETs [37] and is recognized as the most effective. CgA has been recognized as a general serum marker, as it is co-secreted in tumors with the amines and peptides that are present in the neurosecretory granules [38] and can be elevated in both functionally active and nonfunctional NETs. Specificity of elevated CgA is related to tumor type and is almost universally elevated in patients with gastrinoma [39–41]; it is often high in NETs of midgut origin and in nonfunctioning pancreatic NETs. Differences in tumor cell type, histological differentiation and tumor volume may influence the level of CgA and interpretation may also depend on the assay used in measurement.

Reliability of CgA in Patients with NETs

Overall, CgA has been found to be clinically informative and moderately sensitive in the majority of studies devoted to this topic. CgA was found to be more sensitive than neuron-specific enolase (NSE) in all subgroups of a large mixed NET patient cohort ($n = 128$) [42]. While performances have been limited in low-level cutoffs due to the overlap with control populations, very high levels of serum CgA are rarely found outside the setting of NETs with the exception of patients on gastric acid secretory blockers, especially PPIs [43] or those with hypergastrinemia. Specificity of CgA in the diagnosis of NETs depends on the tumor type and burden (100% specificities have been reported in patients with metastatic disease [44–47]), the quality of the control populations used and the cutoff values employed [40, 48]. Elevated CgA was found to be more sensitive than high U-5-HIAA levels in patients with metastatic midgut lesions (87 vs. 76%, respectively) [4]. Nobels et al. [39] demonstrated a significant positive relation between the serum levels of CgA and the tumor mass in NETs; however, the distinction between high and low tumor volume may be open to question. This study also confirmed tissue specificities as high CgA concentrations were found in all patients with gastrino-

ma, although small in size and tumor volume [39]. In a mixed series of 128 patients with NETs, increased CgA levels were found in 29 and 67% of patients with locoregional or metastatic disease, respectively [42]. A prognostic value of CgA in patients with NET has not been reported in several studies [4, 49, 50].

False-Positive Elevation of CgA May Occur in the Following Circumstances

- Impaired renal function [51, 52]
- Parkinson disease, untreated hypertension and pregnancy
- Steroid treatment or glucocorticoid excess, which can lead to upregulation of CgA mRNA [53, 54]
- Chronic atrophic gastritis (type A) [55]
- Treatment with antisecretory medications, especially PPIs [43]

Chronic elevation of gastrin levels provokes hyperplasia of the neuroendocrine cells of the stomach and these cells are able to secrete CgA [56]. In patients with chronically elevated CgA and ZES, it was demonstrated that the CgA concentrations can be normalized by gastrectomy alone, without resection of the gastrin-producing tumor [57]. A more recently described case report of false-positive CgA was due to the presence of heterophile antibodies (HAb), which can bind to animal antigens and may be present in up to 40% of the normal population [58]; in the CgA immunometric assays, HAb interferences may be circumnavigated by using an HAb-blocking tube [59].

Assays for CgA

A recognized international standard for CgA assay is not available and variations in assay types may influence results. Several assays for measurements of intact CgA and cleavage products have been developed [38, 60]. The complexity of assays is explained by the presence of several CgA-related peptides from human and other species [61] and CgA processing varies according to neuroendocrine cells/tissues [62, 63]. A competitive radioimmunoassay can detect circulating CgA, with the use of purified full-length human CgA [45, 64]. Commercial CgA kits differ in the types of antibodies used (monoclonal vs. polyclonal) and include enzyme detection (ELISA) and radioimmunoassay. Differences in methods of standardization have also led to heterogeneity. Generally, measurement of intact CgA in plasma has greater sensitivity for the diagnosis of NETs than the measurement of fragments [38, 65]. Stridsberg et al. [48] compared the 3 commercially available kits in a group of NET patients and found sensitivities to vary between 67 and 93%, while

specificities were >85% for all 3. A recent multicenter prospective comparison between 2 methods, immunoradiometric and ELISA, found a 36% clinical discordance rate [66]. These results were mirrored with a difference of 5-fold interlaboratory variation rate in a recent Italian study aimed at assessing CgA detection performance as applied to immunoradiometric and ELISA assays [67]. A further prospective analysis underlined that CgA is a practical marker in patients with NETs, however, with limited diagnostic power; using ROC curves, a cutoff of 53 ng/mL for IRMA and 16 U/L for ELISA for discriminating between healthy controls and NET patients yielded only moderate sensitivities (71.3 and 83%, respectively) and specificities (71 and 85%, respectively) [47].

General Remarks on CgA

- CgA is the most practical and useful general serum tumor marker in patients with NETs.
- Elevated CgA can occur in normal individuals and in patients with non-NETs although the levels are usually lower than in patients with NETs.
- Sensitivity of elevated CgA varies according to NET tumor type and volume.

CgA Assays and Interpretation

- Reference laboratories should be preferred for clinical samples assays.
- Reference intervals and individual patient results differ significantly between different CgA assays and cannot be directly compared.
- Serial measurements should be performed using the same assay.
- If assays are changed, patients should undergo a new baseline measurement.
- False-positive results are possible in patients with hypergastrinemia (especially on antisecretory medications or chronic atrophic gastritis type A) and in the presence of HAbs (care in patients with autoimmune diseases or those sensitized to rodent proteins (mouse monoclonal antibodies)).
- Where possible, PPIs should be interrupted, leaving a clearance of at least 3 half-lives, prior to CgA plasma sampling.

Other and Emerging Biomarkers in Clinical Use

Serum NSE is considered a tumor marker in NETs [39, 68] and is elevated in 30–50% of the patients, particularly in those with high-grade tumors (poorly differentiated

tumors). The prognostic role of NSE as a biomarker has been evaluated as well [69, 70]. Pro-gastrin-releasing peptide has demonstrated clinical benefit in atypical lung carcinoids and other high-grade lung NETs [68, 71].

Pancreastatin (a part of the CgA molecule) is a good marker for gastrointestinal NETs. It has been claimed to be better than CgA; however, there are only few assays available, mainly for preclinical routine use [72].

Neurokinin A has been suggested to be a good/reliable marker in SI NET [73, 74].

In functional tumors, measurements of specific hormones or other biomarkers can be useful. ACTH and cortisol are assessed for the diagnosis and monitoring of NET-associated ectopic Cushing syndrome, whereas PTHrp for hypercalcemia associated with PTHrp-secreting NETs [75].

NT-proBNP is a valid marker in the clinical evaluation of carcinoid heart disease [76].

Blood measurements of neuroendocrine gene transcripts have demonstrated significant diagnostic and prognostic potential in recent studies (NET-test). The precise role of these analyses has to be expected in future prospective trials [77–79].

Finally, circulating tumor cells is another new tool for diagnosing and follow-up of NET patients [80]. However, it also needs to be evaluated in prospective trials.

Appendix

Antibes Consensus Conference Participants

Arnold, R. (Munich, Germany); Bartsch, D.K. (Department of Surgery, Philipps University, Marburg, Germany); Baudin, E. (Département de Médecine, Gustave Roussy Cancer Campus, Paris South University, Villejuif, France); Borbath, I. (Service de Gastroenterologie, Cliniques Universitaires St-Luc, Bruxelles, Belgium); Capdevila, J. (Vall d'Hebron University Hospital, Teknon Institute of Oncology, Barcelona, Spain); Caplin, M. (Neuroendocrine Tumour Unit, Royal Free Hospital, London, UK); Chen, Y.-J. (Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China); Costa, F. (Hospital Sírio Libanês, São Paulo, Brazil); Cwikla, J.B. (Department of Radiology, Faculty of Medical Sciences, University of Warmia and Mazury, Olsztyn, Poland); Davies, P. (Neuroendocrine Tumour Unit, Royal Free Hospital, London, UK); de Herder, W.W. (Department of Internal Medicine, Division of Endocrinology, ENETS Centre of Excellence Rotterdam, Erasmus MC, Rotterdam, The Netherlands); Eriksson, B. (Department of Endocrine Oncology, Uppsala University Hospital, Uppsala, Sweden); Falkerby, J. (Department of Endocrine Oncology, Uppsala University Hospital, Uppsala, Sweden); Fazio, N. (Unit of Gastrointestinal Medical Oncology and Neuroendocrine Tumors, European Institute of Oncology, Milan, Italy); Garcia-Carbonero, R. (Medical Oncology Department, Hospital Universitario Doce de Octubre, Madrid, Spain); Grozinsky-Glasberg,

S. (Neuroendocrine Tumor Unit, Endocrinology and Metabolism Service, Department of Medicine, Hadassah-Hebrew University Medical Center, Jerusalem, Israel); Gorbunova, V. (Department of Oncology, Institution of Russian Academy of Medical Sciences, Moscow, Russia); Hicks, R.J. (Cancer Imaging, the Peter MacCallum Cancer Centre, Melbourne, VIC, Australia); Hörsch, D. (Gastroenterology and Endocrinology Center for Neuroendocrine Tumors Bad Berka, Bad Berka, Germany); Tiensuu Janson, E. (Department of Endocrine Oncology, Uppsala University Hospital, Uppsala, Sweden); Kaltsas, G. (Department of Pathophysiology, Division of Endocrinology, National and Kapodistrian University of Athens, Athens, Greece); Knigge, U. (Neuroendocrine Tumor Center of Excellence, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark); Kos-Kudla, B. (Department of Endocrinology, Medical University of Silesia, Katowice, Poland); Krenning, E.P. (Cyclotron Rotterdam BV, Erasmus MC, Rotterdam, The Netherlands); Kulke, M.H. (Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA); Kwekkeboom, D.J. (Department of Internal Medicine, Division of Nuclear Medicine, ENETS Centre of Excellence Rotterdam, Erasmus MC, Rotterdam, The Netherlands); Lombard-Bohas, C. (Medical Oncology Department, Hôpital Edouard Herriot, Hospices Civils de Lyon, Lyon, France); Niederle, B. (Department of Surgery, Medical University

of Vienna, Vienna, Austria); Nieveen van Dijkum, E.J.M. (Department of Surgery, Academic Medical Center, Amsterdam, The Netherlands); O'Connor, J. (Department of Clinical Oncology, Institute Alexander Fleming, Buenos Aires, Argentina); O'Toole, D. (NET Centre, St. Vincent's University and Department of Clinical Medicine, St James Hospital and Trinity College, Dublin, Ireland); Pascher, A. (Department of Surgery, Charité Universitätsmedizin Berlin, Berlin, Germany); Pavel, M. (Department of Hepatology and Gastroenterology, Campus Virchow Klinikum, Charité Universitätsmedizin Berlin, Berlin, Germany); Ramage, J. (Gastroenterology Department, Hampshire Hospitals NHS Trust, Hampshire, UK); Reed, N. (Beatson Oncology Centre, Gartnavel General Hospital, Glasgow, UK); Rinke, A. (Division of Gastroenterology and Endocrinology, University Hospital Marburg [UKGM], Marburg, Germany); Sorbye, H. (Department of Oncology, Haukeland University Hospital, Bergen, Norway); Sundin, A. (Department of Radiology, Institute Surgical Sciences, Uppsala University, Uppsala University Hospital, Uppsala, Sweden); Toumpanakis, C. (Neuroendocrine Tumour Unit, Royal Free Hospital, London, UK); Valle, J.W. (Department of Medical Oncology, The Christie NHS Foundation Trust, University of Manchester/Institute of Cancer Sciences, Manchester, UK); Vulliamme, M.-P. (Service de Gastroentérologie, Hôpital Beaujon, Clichy, France).

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